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RESPIRATORY TRACT CARCINOGENESIS INDUCED BY RADIONUCLIDES IN THE SYRIAN HAMSTER

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ABSTRACT

Syrian hamsters were exposed to lung irradiation by various modalities that have differed in degree of localization and the fraction of lung exposed. The animals have been given alpha emitters under several exposure conditions: intratracheal (IT) instillation of ^{210}Po and $^{239}PuO_2$-ZrO_2 microspheres, inhalation (INH) of ^{238} & $^{239}PuO_2$-ZrO_2 particles and/or intravenous (IV) injection of ^{238} & $^{239}PuO_2$-ZrO_2 microspheres. Beta-emitting ^{147}Pm was also administered, using our standard IV injection technique in which the radionuclide is incorporated into 10 μm diameter ZrO_2 microspheres and deposited in the lungs via the jugular vein. These microspheres lodge quantitatively in the pulmonary capillary bed for the duration of the animal's life span. Total IV Pu microsphere lung burdens have ranged from 0.14 nCi to 484 nCi and the number of spheres from 1500 to 880 000. Pu burdens from inhalation have ranged from 8 nCi to 101 nCi, IT Po burdens from 25 to 122 nCi, and Pm-laden microsphere burdens from 427 to 15 750 nCi.

Intratracheal instillation of ^{210}Po solution gave nearly uniform alpha irradiation of the entire lung, intravenous injection of large numbers of ZrO_2 microspheres laden with ^{147}Pm gave whole lung exposures to low LET radiation, and IV injection of Pu-microspheres provided a gradation of focal alpha exposures. The Po and Pm exposures were highly tumorigenic, whereas the Pu microspheres produced tumors only when a large fraction of the lung was exposed to large radiation doses. However, Pu-ZrO_2 administered via inhalation was very carcinogenic and resulted in tumor incidences of 50% in some experiments.

The IT instillation of Fe_2O_3 following IV or IT Pu-ZrO_2 microsphere administration dramatically enhanced lung tumor induction.

INTRODUCTION

In the late 1960's, the Los Alamos Scientific Laboratory (LASL) embarked on a program to delineate the role of internally deposited radiation in carcinogenesis of the respiratory tract. Initially the principal concern was to study the comparative tumorigenicity of localized versus diffuse alpha irradiation in the hamster using 10 μm diameter ceramic microspheres of PuO_2-ZrO_2 lodged in the pulmonary capillary bed. The purpose of this approach was to determine whether radiation from localized "hot spots" was more hazardous than an equivalent amount of radiation uniformly distributed throughout the entire lung. It was generally believed at the time that dose localization probably would decrease rather than increase tumor risk (Bair et al., 1974; Nat. Acad. Sci., 1976; Commission on Radiat. Protection, 1978). LASL's endeavor was conceived by W. H. Langham and C. R. Richmond, and calculations by Dean and Langham, (1969) suggested that if the dose-response curve for rat skin (Albert et al., 1967) approximated that for single cells in the pulmonary milieu, a relatively small number of plutonium-containing particles deposited in the lungs of animals should elicit a significant number of primary tumors. The Syrian golden hamster was selected because of its low spontaneous lung tumor incidence, freedom from infectious chronic respiratory disease (CRD) and susceptibility to both chemical carcinogenesis of the respiratory tract (Saffiotti et al., 1968) and radiation-induced tumorigenesis in the lung (DeVilliers and Gross, 1966; Warren and Gates, 1968; Little et al., 1970). The "hot particle" studies were extended to include low-LET radiation under carefully defined conditions of dose rates and fraction of lung exposed. ^{147}Promethium, a soft β emitter, was incorporated into the ZrO_2 ceramic microspheres and lodged in the pulmonary capillary bed via jugular vein injection (Anderson et al., 1979b).

Finally, attempts were made to address other fundamental problems associated with radiation-induced respiratory carcinogenesis such as the role of enhancing agents and the effects of particle composition and chemical matrix on inhaled radionuclide-induced tumor development. This report summarizes the results obtained from this multidisciplinary approach.

MATERIALS AND METHODS

Animals

Syrian (golden) hamsters (Mesocricetus auratus) were obtained from either the Lakeview Hamster Colony (Newfield, NJ) or Engle Laboratory Animals (Hammond, IN) at an age of 4 to 6 weeks and were admitted to our experimental animal colony after a 2-week quarantine. Exposed or control animals were housed two to a polycarbonate cage with aspen shavings (low dust) for bedding. A sheet of DuPont No. 22 spinbonded polyester filter covered each shelf and minimized contamination between individual cages. Cages and bedding were changed twice weekly, and animals were inspected daily. Water and a commercial stock diet (Teklad Hamster Diet, Teklad Mills, Winfield, IA) were provided ad libitum. Water bottles were equipped with disposable caps and autoclaved sipper tubes. The animals were exposed at an age of about 100 days after at least 50 days of conditioning at Los Alamos altitude (2200 m).

When anesthesia was necessary (IV microsphere injections and IT instillations of Fe_2O_3/saline), the hamsters were given intramuscular (IM) ketamine hydrochloride (Ketaset, Winthrop Laboratories, New York, NY). To inject the spheres, a jugular vein was exposed and isolated by sharp and blunt dissection and the spheres, suspended in saline, were flushed into the vein (Holland et al., 1971). The animals were suspended vertically against a plexiglass stand with their mouths held open by a fixed wire and an elastic band for the

IT instillations. A 19 gauge, 7 cm blunt-tipped cannula was used to instill the hematite/saline at the tracheal bifurcation (Smith et al., 1974).

After injections, all animals were checked daily, moribund hamsters were killed, and dead animals were necropsied as soon as feasible. The respiratory tract was instilled via the trachea with 5 to 6 ml of 10% neutral buffered formalin and the trachea occluded by clamping with forceps. The trachea, lung, and heart were removed en bloc and fixed in formalin.

Complete gross and microscopic examinations were performed on all organs. For histology, right and left lungs were separated after fixation. The left lung was bisected along the rostral-caudal axis parallel to the main stem bronchus, and both portions were embedded in a single block. The right lung was embedded in a separate block. The blocks were sectioned at 6 μm and routinely stained with hematoxylin and eosin. Lung tumors and other lesions were scored by scanning multiple sections that included longitudinal sections of major bronchi. All results were stored in a computerized data base for retrieval and analysis.

Microspheres

Production and properties of the IV injected microspheres have been described in detail (Anderson and Perrings, 1978). The spheres consisted of a high-fired (1000° C) ceramic of ZrO_2 containing a small fraction (9×10^{-5} to 1.1×10^{-2}) of PuO_2 or, in a few experiments, the β-emitter ^{147}Pm. Measurements of the distribution of sphere volumes with a Coulter counter (Coulter Electronics, Hialeah, FL) gave a coefficient of variation of 4% (corresponding to 1.3% diameter resolution). The variability in Pu content, determined by counting α radiation of individual spheres from the most active batches, was found to be ± 2.6%. The amount of Pu added to the various batches of spheres was varied within wide limits (0.07 to 59 pCi/sphere) to

determine which specific particle activity was most tumorigenic. Particles of pure PuO_2 with these activities would have diameters of 0.09 to 0.86 μm for ^{238}Pu and 0.6-5.6 μm for ^{239}Pu, thus approximating the size range of respirable particles. The isotope actually used in microspheres of a given activity was governed by the desire to keep the chemical concentration of the Pu below 1%. Thus, ^{239}Pu was used in the spheres with activities below 2 pCi and ^{238}Pu in spheres with higher activities. Because the spheres were predominantly ZrO_2, all specific activities were far below those of similarly sized pure PuO_2 particles, and effects (such as increased apparent solubility) associated with the very high specific activity of $^{238}PuO_2$ did not occur. The only difference between the two isotopes is the greater energy and range of the ^{238}Pu α particles. The number of α microspheres injected ranged from 0 (controls) to 880 000 yielding lung burdens of 0 - 484 nCi (Holland et al., 1976; Smith et al., 1976; Anderson et al., 1979a). To facilitate the in vivo measurements of injected and retained doses, a low-level tag of ^{57}Co was added to all spheres. Cobalt-57 decays by pure electron capture with the emission of γ rays of energy 122 and 136 keV. The amount added (typically 1 to 2 pCi/sphere) gave a γ dose to the lung 1/300 that from the Pu α spheres on a rad basis. For IV injection, the desired number of microspheres (verified by ^{57}Co counting) was suspended in saline, and drawn into a 40 cm length of polyethylene tubing 0.58 mm inner diameter. The contents of the tubing were injected into the jugular vein by flushing with 0.5 ml of 0.15 m NaCl from a pulsed-flow dental hygiene device (Holland et al., 1971). The pulsations are necessary to keep the dense spheres in suspension and to obtain quantitative delivery. Detailed retention studies showed no detectable excretion or translocation. The average coefficient of variation of

individual hamster doses about their mean was 28%. The actual number of spheres given each animal was recorded.

Results obtained with ß-emitting microspheres containing ^{147}Pm are summarized here (Table I). Details are reported elsewhere (Anderson et al., 1979b).

Polonium-210 Instillations

In the experiment with ^{210}Po, IT instillation under sodium methohexital anesthesia was performed according to the procedure established by Little and Kennedy, (1979). Each animal was given 0.2 ml of freshly prepared solution (10 µl of a 1 M HNO_3 solution of Po in 10 ml of physiological saline). The dose was estimated by liquid scintillation counting of "dummy dose" aliquots and was given weekly for 7 or 15 weeks. No ferric oxide was added as carrier, and the hydrolyzed Po was freshly dispersed at administration.

Fe_2O_3/Saline Instillations

One week following either the IT or IV administration of microspheres (Table III), the hamsters were given either 0, 1 or 10 (once a week x 10 weeks) doses of 0.3 mg or 3 mg Fe_2O_3 in 0.2 ml saline IT. Fe_2O_3 (certified anhydrous, Fisher Scientific Co., Fair Lawn, NJ) was suspended in 0.15 M NaCl via sonification with a sonifier cell disruptor (Heat Systems Co., Melville, NY).

Inhaled PuO_2-ZrO_2

Details for this facet of our work are given elsewhere (Thomas and Smith, 1979a, 1979b). Briefly, groups of hamsters were divided and exposed as shown in Table IV. In addition to those animals used to assess the incidence of tumorigenesis, a few were killed to establish tissue distributions of the radionuclide. The animals with combined INH and IV administrations received the injected microspheres one week prior to the inhalation. The "nose-only"

inhalation chamber and aerosol generation system have been described (Raabe, et al., 1973). The starting material in the nebulizer (Mercer, et al., 1968) was zirconium dioxide sol identical to that used in the process of manufacturing microspheres for IV injection (Anderson and Perrings, 1978). The desired amount of ^{238} or $^{239}Pu(NO_3)_4$ was added to the sol plus a small amount of ^{57}Co gamma emitting tracer. The droplets were passed through a heating column at 900°C before entering the inhalation chamber and the animals were exposed "nose-only" for periods of approximately 20 minutes.

All lesions were classified morphologically according to descriptions given elsewhere (Smith et al., 1974, 1976; Thomas and Smith, 1979a.

RESULTS

A summary of the "hot particle" data is provided in Table I, in which the experiments are tabulated into three categories: controls, localized, and diffuse exposures. Control animals received either no spheres, microspheres that contained no radioactivity or those that had only the ^{57}Co tag (about 1.6 pCi/sphere). Three of the control animals, from a group of 88 that had received 106 000 spheres containing only the ^{57}Co tag, were the only controls to develop lung tumors. This is the only experiment in our program in which animals receiving only the ^{57}Co-tagged spheres developed tumors. Thus, it is difficult to ascribe tumorigenicity to ^{57}Co γ irradiation at these levels. The overall incidence of bronchiolar adenomatoid lesion (BAL) proliferation of terminal bronchiolar epithelium into alveoli that is considered by some to be a preneoplastic change, in control animals was 14%.

Animals in the "localized exposure" category were given α-emitting microspheres IV in such numbers (1500 to 15 000) that less than 10% of the lung mass was irradiated focally. This minimized overlapping of individual

TABLE I
Summary of LASL "Hot Particle" Data

	No. of Spheres	Nuclide	Lung Burden (nCi)	No. of Hamsters	Lung Tumor Incidence	Malignant Lung Tumor Incidence	BAL Incidence[a]
Controls	0-1M	---	0	521	0.6% (0-3%)	0.2%	14%
Localized Exposures (α-spheres)	1.5-15K	^{238}Pu or ^{239}Pu	0.14-484	862	0.8% (0-4%)	0.5%	4%
Diffuse Exposures (α-spheres)	34-880K	^{238}Pu or ^{239}Pu	31-131	307	6.8% (1-12%)	2.3%	20%
Diffuse Exposures (β-spheres)	6-152K	^{147}Pm	427-15 750	239	20% (0-38%)	13%	30%
Diffuse Exposures (IT α solution)	---	^{210}Po	25-122	155	44% (39-50%)	19%	---

[a] Bronchiolar Adenomatoid Lesion in nontumor-bearing animals.

radiation fields insuring that exposure conditions would be truly from focal point sources. The activity of individual spheres was from 0.07 pCi. [the minimum activity originally defined by Tamplin and Cochran (1970) as being a "hot particle"] to 59pCi. Seven of the 862 animals at risk developed primary lung tumors and the BAL incidence was 4%. Total lung burdens in diffuse alpha microsphere exposures were from 31-131 nCi which were calculated to irradiate from 17% to greater than 99% of the lung mass. Numbers of microspheres injected IV were 34 000 to 860 000. The primary lung tumor incidence was 6.8% with 2.3% having malignant adenocarcinomas and 20%, BAL. Tumorigenecity of alpha emitting microspheres versus fraction of lung irradiated is given in Table II. Significant numbers of lung tumors were not produced until 28% of the total lung was exposed to the alpha-irradiation.

Animals receiving beta-emitting (^{147}Pu) spheres were given large numbers, 6000 to 152 000, so that lung burdens ranged from 427 - 15 750 nCi, irradiating 100% of the lung at the higher levels. The mean lung tumor incidence in these studies was from 0 - 38% (Anderson et al., 1979b). Tumors were not produced until the lung burden was 3360 nCi. The BAL frequency in these animals was 30%.

Lung burdens from the instillation of solutions of ^{210}Po were from 25 to 122 nCi which resulted in an overall tumor incidence of 44%; 19% of the animals had malignant tumors of the respiratory tract, usually mixed adenosquamous carcinomas, that arose peripherally in the bronchial tree.

Details of the exposure regimen for the studies using Fe_2O_3/saline as enhancing agents to alpha-induced lung tumor development are presented in Table III. Tumor incidences are likewise summarized in Table III which gives the ratio of animals with tumors to total animals. The standard deviations (SD) were calculated for binomial statistics ($\sigma^2 = npq$), where n = number of

TABLE II
Tumorigenicity of Alpha Microspheres

Number of Spheres/Animal	Fraction of Lung Irradiated (%)	Microsphere Specific Activity (pCi)	Lung Burden (nCi)	Tumors/ Animals	Tumor Incidence (% ± SD)
2,360	1	58.9	139	0/68	0 ± 2
10,900	5	8.9	97	0/17	0 ± 6
58,800	28	2.0	118	19/160	12 ± 3
312,000	80	0.4	131	2/25	8 ± 6

TABLE III
Effect of Ferric Oxide on Tumor Induction by Microspheres

	Animals with Tumors/Total Animals	
	IV Spheres	IT Spheres
No Ferric Oxide	1/66 = 2 ± 2%[a]	4/73 = 5 ± 3%[b]
Ferric Oxide (3 mg once)	0/27 = 0 ± 3%	8/21 = 38 ± 11%
Ferric Oxide (0.3 mg 10 times, weekly; 3 mg total)	7/41 = 17 ± 6%	13/33 = 39 ± 9%
Ferric Oxide (3.0 mg 10 times, weekly; 30 mg total)	1/24 = 4 ± 4%	18/35 = 51 ± 8%

[a]Total burdens: 33 nCi (30 000 spheres x 1.1 pCi/sphere), 17 animals; 45 nCi (50 000 spheres x 0.9 pCi/sphere, 21 animals; and 54 nCi (60 000 spheres x 0.9 pCi/sphere), 28 animals.

[b]Total burdens: 33 nCi (30 000 spheres x 1.1 pCi/sphere), 17 animals; and 76 nCi (36 000 spheres x 2.1 pCi/sphere), 56 animals.

animals, p = fraction with tumors, and q = fraction without tumors). Approximately one-third of the tumors were adenocarcinomas and two-thirds adenomas with induction times of 25 to 82 wk. Control animals receiving Fe_2O_3 alone once a week for 10 wk did not develop tumors (0/18 = 0 ± 5%)(data not shown). Animals receiving IV spheres alone had a primary lung tumor incidence of 2 ± 2% and those given IV spheres and a single instillation of Fe_2O_3 0 ± 3%. Ten instillations of Fe_2O_3 increased the lung tumor incidence to 7/41, 17 ± 6% for 0.3 mg/wk, and 1/24, 4 ± 4% for 3.0 mg/wk. When microspheres were given IT, 4/73 (5 ± 3%) hamsters developed lung tumors. Even a single Fe_2O_3 instillation raised the tumor incidence to 8/21 (38 ± 11%) -- in marked contrast to the lack of effect with IV spheres. Ten repeated instillations at two different levels did not result in a significant additional increase in tumor incidence (13/33, 39 ± 9% for 0.3 mg/wk, and 18/35, 51 ± 8% for 3.0 mg/wk). It is concluded from these results that IT instillation of Fe_2O_3 enhanced tumor induction by both IV and IT Pu/ZrO_2 microspheres, that enhancement was greater for IT spheres, and that IT spheres may be somewhat more tumorigenic than IV spheres in the absence of hematite enhancement.

The experimental design for our studies with $(Pu-Zr)O_2$ injected microspheres and/or inhaled particles by Syrian hamsters is included as Table IV. The incidence and type of pulmonary tumors and BAL incidence are given in Table V. The highest frequency of lung tumors (50%) occurred in Group I animals which received mean initial long-term lung burdens of 101 nCi ^{238}Pu via inhalation only. Group G with a mean total lung burden of 143 nCi ^{238}Pu (56 nCi via 20 000 microspheres IV plus 87 nCi via inhalation) had a 40% lung tumor incidence. Twenty-eight percent of the hamsters in Group H developed pulmonary tumors. They had a mean total lung burden of 129 nCi ^{238}Pu achieved with 53 nCi from 20 000 microspheres IV and 76 nCi via

TABLE IV
Experimental Design for Studies with $(Pu\text{-}Zr)O_2$
Injected IV into and/or Inhaled by Syrian Hamsters

Group ID	Number of Hamsters[a]	Radio-Nuclide	Mean # Spheres Injected	Mean Initial Pu Lung Burdens (nCi)[b]	
				Injection	Inhalation
A	53	^{239}Pu	~ 60 000	117	0
B	43	^{239}Pu	0	0	8
C	40	^{239}Pu	~ 30 000	49	6
D[c]	45	--	~ 30 000	0	0
E[d]	55	--	0	0	0
F[e]	44	--	0	0	0
G	50	^{238}Pu	~ 20 000	56	87
H	60	^{238}Pu	~ 20 000	53	76
I	44	^{238}Pu	0	0	101
J	45	--	0	0	0

[a]Does not include serially killed animals out to 32 days postexposure.

[b]Estimated from long term lung retention kinetics (alveolar burden). The two digits used in these numbers were determined from retention equations, but it is obvious from such techniques that rounding off is a sufficiently accurate accounting of the actual lung burdens (e.g. 87 nCi could as accurately be reported as 90 nCi).

[c]Group D received ^{57}Co labeled ZrO_2 microspheres IV and aerosol particles.

[d]Group E received ^{57}Co labeled ZrO_2 aerosol particles.

[e]Group F received unlabeled ZrO_2 aerosol particles.

TABLE V
Pulmonary Neoplasms and BAL Incidence

Group	# Tumor Bearing Animals ÷ # of Animals	# with Multiple Tumors ÷ # with Tumors	Adenoma	Squamous Adeno-Carcinoma	Squamous Cell Carcinoma	BAL Incidence[a]
A	1/53 (2%)	0/1 (0%)	1/1 (100%)	--	--	24/52 (46%
B	5/43 (12%)	0/5 (0%)	5/5 (100%)	--	--	4/38 (11%)
C	2/40 (5%)	0/2 (0%)	2/2 (100%)	--	--	7/38 (18%)
D	0/45 (0%)	--	--	--	--	1/45 (2%)
E	1/55 (2%)	0/1 (0%)	1/1 (100%)	--	--	5/54 (9%)
F	0/44 (0%)	--	--	--	--	5/44 (11%)
G	20/50 (40%)	4/20 (20%)	12/20 (60%)	8/20 (40%)	--	18/30 (60%
H	17/60 (28%)	3/17 (18%)	11/17 (65%)	6/17 (35%)	--	11/43 (26%
I	22/44 (50%)	4/22 (18%)	10/22 (45%)	9/22 (41%)	3/22 (14%)	12/22 (55%
J	0/45 (0%)	--	--	--	--	1/45 (2%)

[a] In nontumor-bearing animals.

inhalation. The predominant tumors in G, H, and I were adenomas; however, adenocarcinomas were prevelant in all three groups. The only squamous cell (epidermoid) carcinomas found in this study were in Group I. The vast majority (~ 90%) of all tumors were peripheral, i.e., arising from the bronchial tree in or distal to condary bronchi with the notable exception of Group I's squamous cell carcinomas which originated from bronchial epithelium in hilar portions of the lung. Those Groups, A, D, E, F, and J, that received microspheres without inhaled particles had extremely low or a zero incidence of pulmonary neoplasms. Interestingly, Group B, which received only 8 nCi ^{239}Pu via inhalation, and Group C which was given 30 000 microspheres IV (49 nCi ^{239}Pu) plus 6 nCi inhaled ^{239}Pu, had lung tumor incidences of 12% and 5% respectively. It is obvious from Tables IV and V that the major factor resulting in lung tumor growth and development in this study was the administration of ^{239}Pu or ^{238}Pu via inhalation. Apparently, microspheres containing ^{239}Pu lodged intravascularly had little or no influence in tumorigenesis.

BAL occurred at an unusually high and unexpected frequency in Group A considering the low number of tumors observed in this group, and the lower incidence obtained in earlier studies (Smith, et al., 1976). Further, since this lesion was also found in hamsters that received no radiation (Groups E, F, and J), it is difficult to define this change as definitively being preneoplastic.

To conclude, significant numbers of primary lung tumors were induced in Syrian hamsters that received plutonium-zirconium dioxide via inhalation. The addition of radiation administered via plutonium laden ceramic microspheres

IV which lodged in the lung capillary beds had little effect on tumor production. Radiation emitted from plutonium particles deposited in the respiratory tract after inhalation was the major factor in tumorigenesis.

DISCUSSION

Under the experimental conditions prevailing in our laboratory, isolated sources of intense alpha irradiation (microspheres) repeatedly have been very ineffective in inducing lung tumors. Microspheres induce significant numbers of lung tumors only when large portions of the lung mass are irradiated (Table II). When the activity is confined to smaller numbers of particles, there is no detectable tumorigenicity. Thus the greater hazard is present when the radiation is more uniformly distributed throughout the pulmonary milieu.

The results indicate that the hamster is not insensitive to tumor induction by beta radiation in the form of ceramic microspheres lodged in the lung parenchyma (Anderson *et al.*, 1979b). Our ^{147}Pm response is similar to that of a number of low-LET radiations in several species as summarized by Bair *et al.*, (1974). The primary features are the (1) high doses required to produce a modest tumor incidence (10^4 rad giving 10 - 30% tumors), (2) insensitivity of the response to large increases in dose (15 - 20% increment for a decade of dose increase), and (3) lack of indication of declining incidence even at very high cumulative doses.

The ^{210}Po exposures confirm the extensive studies of Little and co-workers (1979) and demonstrate the high tumorigenicity (35 - 50%) of this modality of exposure.

The discovery that adhering chemical carcinogens to ferric oxide (Fe_2O_3, "hematite") particles dramatically increases the respiratory tract tumor

incidence laid the foundation for modern lung carcinogenesis studies (Saffiotti, et al., 1964, 1968, 1972). It was thought initially that Fe_2O_3 particles served merely as "inert" carriers facilitating carcinogen transport across bronchial epithelium. However, more recent work suggested that Fe_2O_3 is in itself cocarcinogenic (Cresia and Nettesheim, 1974). Steinback et al., (1973) found that several dusts, MgO, Al_2O_3, and C, given IT, acted synergistically with diethylnitrosamine injected subcutaneously in the induction of respiratory tract tumors in Syrian hamsters.

The role of intratracheally instilled saline in this enhancement remains to be elucidated. We have addressed this question and the results are oncoming. Relationships between amounts and varying dose schedules of Fe_2O_3 and benzo(a)pyrene (BaP) and subsequent respiratory tract tumor induction have been reported by Saffiotti et al., (1972). One possible mechanism for Fe_2O_3 tumor enhancement, other than concentrating carcinogens and delaying their clearance from the respiratory tract (Cresia & Nettesheim, 1974), is that, when it was administered alone IT, cell turnover as evidenced by ^3H-thymidine incorporation into respiratory tract epithelium was increased by a factor of 16 over controls 24 h after injection (Nettesheim, 1972). Anything that increases DNA synthesis has the proclivity of acting as a cocarcinogen. However, Little et al., (1975) found no marked difference in the tumorigenicity of IT ^{210}Po sols with and without Fe_2O_3, suggesting that alpha radiation given diffusely may induce respiratory tumors via different mechanisms than chemical carcinogens.

With the inhalation exposure experiments, two factors were introduced that varied from the "hot particle" concept; namely, the aerosol particles were much smaller and more numerous than the microspheres, and were in motion and actively transported out of the lung. Both of these factors greatly

reduced localized concentration of dose and resulted in a much more diffuse deposition of energy. However, they do not differ in these respects from aerosols of PuO_2, which are also non-tumorogenic in hamster lung.

Results of these studies were surprising in that the inhalation of PuO_2 particles alone had not indicated a tumorigenicity in the Syrian hamster in our laboratory, nor in at least two other laboratories (Hobbs et al., 1976; Sanders, 1977). The obvious questions now being answered through further study, center about the reproducibility of the results and the possible adjunctive role of the ZrO_2 matrix in tumor formation. This latter factor is a real enigma because of the presumed biological inactivity of a ZrO_2 particle, as evidenced by the lack of foreign body reactions or other lesions when the ZrO_2 particles are administered alone without radioactivity. Experiments are in progress substituting thoria and urania for zirconia to determine whether a similar effect exists. These materials are of interest because, in addition to being chemically similar to ZrO_2, they compose the fuel elements of breeder reactors. Results of these further investigations are presently becoming available.

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